Microbial Pathways for the Reduction of Mercury in Saturated Subsurface Sediments

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ABSTRACT

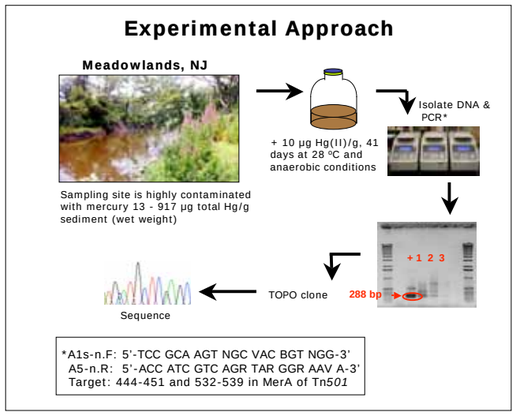
The reduction of inorganic mercury (Hg[II]) to elemental mercury (Hg[0]) may increase the mobility of mercury in ground water and the vadose zone because the interactions of Hg(0) with ligands and available surfaces are weakened. Two pathways for Hg(II) reduction are investigated: (i) an inducible reduction by the bacterial mercury resistance (*mer*) system, and (ii) constitutive reduction by mercury sensitive metal reducing anaerobic bacteria. The hypothesis that is tested by this project is that **under anoxic conditions *mer*-mediated reduction occurs in highly contaminated sediments whereas in environments impacted by low concentrations of mercury the second process dominates**.

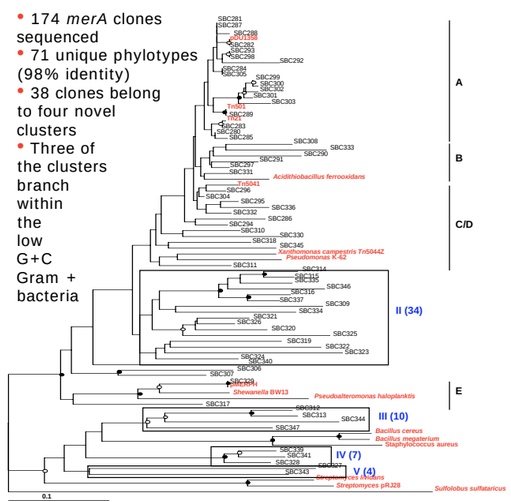
To test this hypothesis we are examining Hg(II) reduction to Hg(0) in incubations of subsurface sediments that are set under varied respiratory conditions with high and low concentrations of Hg(II). The taxonomic composition and presence and expression of *mer* genes are examined in active microbial communities using state-of-the-art molecular analyses in order to assess what pathways dominate under the various incubation conditions. A microbial community from highly contaminated sediment (100's µg Hg/g sediment) that was incubated under fermentative conditions had a high diversity of *merA*, the gene encoding for the central function of the *mer* operon, the mercuric reductase. Many of these genes belonged to novel clusters in the *merA* gene tree, possibly representing reductases that have specialized in reduction of Hg(II) during anoxia.

In less contaminated environments (ng Hg/g) metal reducing anaerobic bacteria, whose activities immobilize radionuclides and toxic metals, also reduce Hg(II). This activity was constitutive and occurred while pure cultures of metal reducers grew with fumarate or iron as terminal electron acceptors. However, specific reduction rates were at least three times higher under iron reducing conditions suggesting that, as has been shown for other oxidized elements, Hg(II) may be reduced indirectly by ferrous iron that is produced during iron reduction. If so, more than one pathway for the constitutive reduction of Hg(II) may take place in environments with low levels of mercury contamination. This study is the first attempt to examine how microbial activities control the mobility of mercury in saturated subsurface sediments and should lead to an improved management practices that will minimize the groundwater contamination.

Fig. 1: NJ tree of derived amino acid sequences from *merA* PCR products (~244 bp) obtained from Berry's Creek sediment. Numbers in parentheses following clade designations indicate the number of clones within that clade. Unique clades (II – V) detected in this library are outlined in boxes.

High diversity and novel clusters in a *merA* clone library from mercury contaminated sediments





New PCR primers that encompass the known diversity of MerA among prokaryotes

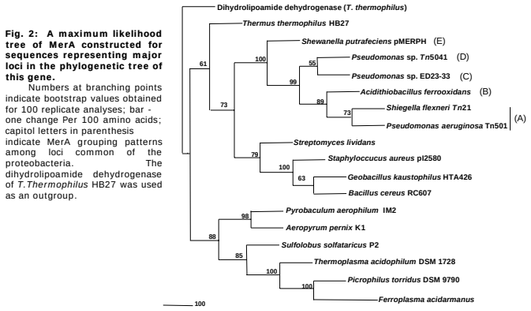
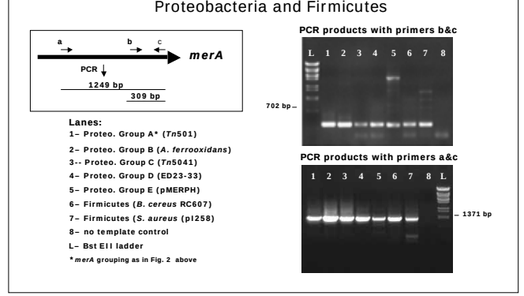
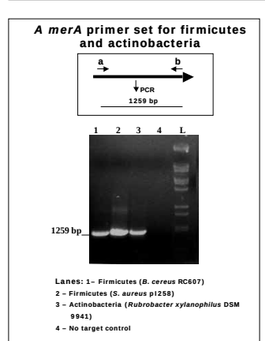
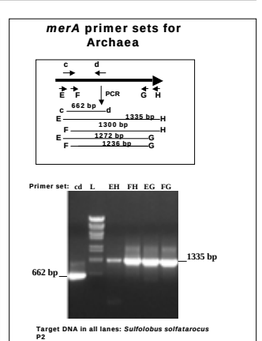


Fig. 2: A maximum likelihood tree of MerA constructed for sequences representing major loci in the phylogenetic tree of this gene. Numbers at branching points indicate bootstrap values obtained for 100 replicate analyses; bar - one change Per 100 amino acids; capitol letters in parenthesis indicate MerA grouping patterns among loci common of the proteobacteria. The dihydrolipoamide dehydrogenase of *T.Thermophilus* HB27 was used as an outgroup.

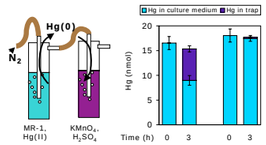






Reduction of Hg(II) by dissimilatory metal reducing bacteria

Fig. 3: *Shewanella oneidensis* MR-1 reduces Hg(II) to Hg(0)

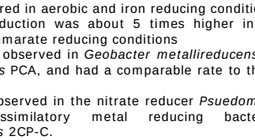


Recovery of Hg at 3h

	Medium		Trap	
	in solution	sorbed to glass	in solution	sorbed to glass
live	42.7 ± 5.1%	11.5 ± 1.1%	32.4 ± 4.3%	3.8 ± 0.9%
heat killed	90.4 ± 2.9%	6.4 ± 1.6%	1.1 ± 1.0%	0.3 ± 0.5%

- MR-1 does not have a *mer* operon, and is not resistant to Hg(II)
- Reduction of Hg(II) by MR-1 occurs at low levels of Hg(II), and is constitutive

Fig. 4: Electron donors and electron acceptors are required for Hg(II) reduction by MR-1



Additional findings:

- Reduction of Hg(II) also occurred in aerobic and iron reducing conditions
- Specific activity of Hg(II) reduction was about 5 times higher in iron reducing conditions relative to fumarate reducing conditions
- Reduction of Hg(II) was also observed in *Geobacter metallireducens* GS-15 and *Geobacter sulfurreducens* PCA, and had a comparable rate to that of MR-1
- No reduction of Hg(II) was observed in the nitrate reducer *Psuedomonas stutzerii* OX-1 or the dissimilatory metal reducing bacterium *Anaeromyxobacter dehalogenans* 2CP-C.

CONCLUSIONS

- **A high, and hitherto unrecognized, diversity of MerA was found in the microbial community of a contaminated anoxic sediment**
- ***merA* primer sets designed and tested**
- **Activities of DMRB in saturated zones may mobilize Hg**
- **These findings and experimental tools are currently used to study Hg mobilization by microbial activities in the subsurface**